Biosynthesis of High Density Lipoprotein by Chicken Liver: Intracellular Transport and Proteolytic Processing of Nascent Apolipoprotein A-1

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ABSTRACT   To study the in vivo processing and secretion of Apolipoprotein A-I (Apo A-I), young chickens were administered individual L-[^{3}H]amino acids intravenously and the time of intracellular transport of nascent Apo A-I from rough endoplasmic reticulum (RER) to the Golgi apparatus was measured. Within 3 to 9 min there was maximal incorporation of radioactivity into Apo A-I in both the RER and the Golgi cell fractions. By contrast, the majority of radioactive albumin was also present in the RER by 3 to 9 min, but did not reach peak amounts in the Golgi fraction until 9 to 25 min. Both radioactive Apo A-I and albumin appeared in the blood at about the same time (between 20 and 30 min). NH$_2$-terminal amino acid sequence analysis of nascent intracellular Apo A-I showed that it contains a pro-hexapeptide extension identical to that of human Apo A-I. After 30 min of administration of radioactive amino acids radioactive Apo A-I was isolated by immunoprecipitation from the liver and serum. NH$_2$-terminal sequence analysis of 20 amino acids indicated that chicken liver contained an equal mixture of nascent pro-Apo A-I and fully processed Apo A-I, whereas the serum only contained processed Apo A-I. Further studies showed that the RER only contained pro-Apo A-I, whereas a mixture of pro-Apo A-I and processed Apo A-I was found in the Golgi complex. These results indicate that, in chicken hepatocytes, there is a more rapid transport of Apo A-I than of albumin from the RER to the Golgi cell fractions, and that Apo A-I remains in the Golgi apparatus for a longer period of time before it is secreted into the blood. In addition these studies show that the in vivo proteolytic processing of chicken pro-Apo A-I to Apo A-I occurs in the Golgi cell fractions.

Apolipoprotein A-I (Apo A-I)[1] is the major protein component of plasma high density lipoprotein (HDL). It is a single polypeptide chain consisting of ~234–243 amino acid residues (2, 6, 21, 25). The structure of plasma Apo A-I has been well studied (8, 11, 17, 18, 20, 22, 35) but the details of its biosynthesis and secretion are still not clear. In human, and most other species, Apo A-I synthesis is thought to occur mainly in the liver and intestine (8, 14, 18, 20, 22, 35), but in avians Apo A-I synthesis also occurs in tissues such as breast,

muscle, and kidney (5, 32). Various aspects of the biosynthesis of Apo A-I in these tissues have recently been investigated (3, 5, 18, 19, 32). Apo A-I, obtained from human intestinal cells in organ culture, occurs as several isoforms, suggesting that it is initially secreted as a basic precursor which is subsequently processed to the more acidic isoproteins found in plasma (39). Gel electrophoretic analysis of the translation products of Apo A-I mRNA demonstrated that Apo A-I is synthesized as a larger precursor (5, 7, 15, 28, 33, 37, 40). NH$_2$-terminal sequence analysis of the in vitro–synthesized Apo A-I showed that it contains a 24-amino acid NH$_2$-terminal polypeptide extension (15). Co-translational cleavage of the in vitro product by ascites and dog pancreas microsomal membranes (16,

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[1]Abbreviations used in this paper: Apo A-1, apolipoprotein A-1; HDL, high density lipoprotein; PMSF, phenylmethylsulfonyl fluoride; RER, rough endoplasmic reticulum; TPCK, N-tosyl-L-phenyl-alanine chloromethyl ketone.

37) indicated that the first 18 amino acids resemble a "signal" prepeptide and that the following hexapeptide is akin to the propeptides seen in several secretory polypeptide hormones. However the propeptide contains carboxy-terminal gln–gln residues and the prosegment is not cleaved intracellularly either by human liver and intestine in organ culture, by perfused rat intestine and liver, or by rat hepatocytes and human hepatoma cells in culture (12, 13, 16, 38, 39, 41).

Nascent apolipoproteins are complexed intracellularly with lipids prior to secretion into the blood. The site of synthesis of the apoprotein, lipid (1, 14), and carbohydrate (34) moieties of lipoproteins have been studied but there is little information on the nature of the nascent lipid protein complexes in the various organelles and on the elapsed time of transfer of nascent Apo A-I from its site of synthesis in the rough endoplasmic reticulum (RER) to the Golgi apparatus. Recently we showed that newly synthesized Apo A-I, even though it is present within the RER and the smooth endoplasmic reticulum, failed to float between densities 1.063–1.21 g/ml, whereas the Apo A-I, which is present within the Golgi complex, is capable of floating at a buoyant density similar to that of plasma HDL (3). Using radioactive glycerol, we have further shown that although glycerol is quickly incorporated into lipids of the endoplasmic reticulum and the Golgi cell fractions, the nascent lipids are mostly conjugated with Apo A-I in the Golgi apparatus (4). This indicates that most of the lipid–protein assembly occurs late in the secretory process, probably in the Golgi apparatus.

In this report we describe the in vivo proteolytic processing of Apo A-I, by measuring the NH_2-terminal amino acid residues of nascent chicken apoprotein A-I in the RER, Golgi apparatus, and plasma; and we show that, compared with albumin, Apo A-I has a distinctive rapid transport time into the Golgi apparatus.

MATERIALS AND METHODS

Materials: L-[5(n)-³H]arginine, L-[G-³H]glutamine, L-[2,3-³H]proline, L-[5-³H]tryptophan, L-[2,5-³H]histidine, L-[4-³H]phenylalanine, L-[2,3-³H]aspartic acid, and L-[4,5-³H]leucine were obtained from Amersham Corp., Arlington Heights, IL. Apotrinin (Trasylol) was purchased from Mobay Chemical Corp., NY; phenylmethylsulfonyl fluoride (PMSF) was from Calbiochem-Behring Corp., La Jolla, CA; and N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and benzamidine were from Sigma Chemical Co., St. Louis, MO. Rabbit immunoglobulin monospecific to chicken serum albumin was purchased from Cappel Laboratories, Cochranville, PA, and young leghorn chickens (5–10-d old) were obtained from Spafas Poultry Farms, Norwich, CT.

Preparation of Apo A-I and Development of Antibodies: Blood was collected from the jugular vein of adult roosters and allowed to clot. The serum was separated from the clot and adjusted to 1 mM EDTA, pH 7.4, and 154 mM NaCl. HDL was floated from the adjusted serum between densities 1.063–1.21 g/ml, dialyzed against 0.15 mM NaCl, 0.01 M sodium phosphate (pH 7.4) at 4°C and delipidated as previously described (3). Rabbit antiserum to HDL apoproteins was prepared and tested for its specificity as previously described (4). This antiserum was used to isolate nascent Apo A-I from the liver and serum.

In Vivo Incorporation of Radioactive Amino Acid into Apo A-I: Nascent Apo A-I was radiolabeled in vivo by injecting individual radioactive amino acids into the jugular veins of young chickens. At various times (from 3 to 60 min), the livers were removed and blood was collected. The livers were rinsed throughly and homogenized in phosphate-buffered saline (10 mM sodium phosphate buffer, pH 7.4, 154 mM NaCl) containing 100 µg/ml PMSF, 100 U/ml trasylol, 1 mM benzamidine, and 1 mM TPCK. When total intracellular Apo A-I was isolated, the homogenate was disrupted by the addition of 0.5% sodium deoxycholate and Triton X-100 and a soluble supernatant fraction was obtained by centrifuging at 105,000 g for 60 min. The Apo A-I or albumin present in this fraction was obtained by immunoprecipitation.

To obtain serum Apo A-I, the blood samples were allowed to clot in the presence of 100 µg/ml PMSF, 100 U/ml Trasylol, 1 mM benzamidine, and 1 mM TPCK for several hours at room temperature. The serum was adjusted to 10 mM sodium phosphate, pH 7.4, 154 mM NaCl, 0.5% sodium deoxycholate, 0.5% Triton X-100, and secreted nascent Apo A-I was isolated by immunoprecipitation.

Preparation of RER and Golgi Cell Fractions: Livers were homogenized in 0.25 M sucrose, filtered through a layer of cheese cloth, and a postmitochondrial supernatant was removed by centrifuging the homogenate at 16,000 g for 10 min. A total microsomal fraction was obtained by centrifuging the post mitochondrial supernatant fraction at 105,000 g for 90 min. The RER and Golgi cell fractions were further separated and characterized as described earlier (4).

Isolation of Radioactive Nascent Apo A-I and Albumin from the RER and Golgi Complex Fractions: The cell fractions were treated with 0.5% sodium deoxycholate, 0.5% Triton X-100 in phosphate-buffered saline (10 mM sodium phosphate, pH 7.4, 154 mM NaCl, 100 µg/ml PMSF, 100 U/ml Trasylol, 1 mM benzamidine, and 1 mM TPCK), and a detergent-soluble fraction was obtained by centrifugation at 105,000 g for 60 min. The radioactive nascent Apo A-I and albumin present in these fractions were recovered by immunoprecipitation and in some experiments also by SDS PAGE.

Immunoprecipitation of Albumin and Apo A-I: Three equal aliquots, each containing 0.5-g tissue equivalent of detergent-soluble fractions prepared from either liver homogenate or isolated organelles, or 0.1 ml of serum prepared as described above, were taken. Each sample received one of the following: (a) 75 µl of antiserum to rooster serum Apo A-I (1 ml antiserum precipitated ~0.15 mg of Apo A-I); (b) 50 µl of antiserum to chicken serum albumin (1 ml antiserum precipitated 0.3 mg of serum albumin); or (c) 75 µl of rabbit preimmune serum. All samples were incubated at 37°C for 60 min, followed by 48 h at 4°C with gentle shaking. The antigen–antibody precipitates were collected by centrifugation and washed several times with 0.02 M sodium phosphate buffer, pH 7.4, containing 154 mM NaCl, 100 µg/ml PMSF, 1 mM benzamidine, 1 mM TPCK, and 100 U/ml Trasylol. The samples treated with preimmune rabbit serum did not contain a precipitate. The immune complex was either (a) suspended in 6 M urea, 1% SDS, and 2% β-mercaptoethanol in 0.01 M Tris-phosphoric acid, pH 6.7, and heated in a boiling water bath for 2 min prior to separation of Apo A-I and/or albumin on SDS PAGE, or (b) dissolved in 200–400 µl aldehyde-free 10% acetic acid. The first method was used when measuring the incorporation of radioactive leucine into Apo A-I and albumin and the rate of transport of these proteins from RER to the Golgi apparatus whereas the second method was applied when radioactive Apo A-I was prepared for amino acid sequencing.

NH_2-terminal Sequence Analysis of Intracellular and Secreted Chicken Apo A-I: Samples of immunopurified Apo A-I that were labeled in vivo by individual radioactive amino acids were mixed with 200 mg polybrene (Beckman Instruments, Inc., Palo Alto, CA) and 50 nmol sperm whale apomyoglobin (Beckman Instruments, Inc.) and subjected to 20 cycles of automated sequential Edman degradation using a 0.1 M Quadrol buffer program in a Beckman 890D Sequencer (Beckman Instruments, Inc.). The phenylthiohydantoin-derivatized amino acids obtained at each cycle were dried under N_2 and their radioactivity measured in presence of 10 ml of Betafluors (National Diagnostics, Inc., Somerville, NJ) using a Packard liquid scintillation counter. The yield in each cycle was obtained by identifying and calculating the amount of amino acids recovered when standard apomyoglobin was used. The derivatized amino acids were separated by high pressure liquid chromatography using a C18 Zorbax ODS column (DuPont Co., Wilmington, DE).

Other Methods: SDS PAGE was performed in 10% polyacrylamide slab gels containing 0.1% SDS using the gel and buffer system described by Laemmli (26). In some experiments, the radioactive proteins were detected by cutting the gels into 0.5-mm slices and determining radioactivity in the excised pieces. The slices were digested at 70–75°C in 0.4 ml 30% hydrogen peroxide and 0.2 ml of 60% perchloric acid for 2 to 4 hr. The samples were cooled at 4°C and counted in presence of 10 ml of Scintiverse II (Fisher Scientific Co., Fair Lawn, NJ) in a liquid scintillation spectrometer. Amino acid composition of Apo A-I was determined in a Beckman amino acid analyzer 6300 using ninhydrin reaction program No. 1 (Beckman Instruments, Inc.).

RESULTS

Time Course of Albumin and Apo A-I Secretion

These initial experiments were designed to establish and compare the time required for secretion of nascent albumin and Apo A-I. Both radioactive proteins appeared in the blood

at about the same time (Fig. 1). In the first 15 min following the administration of L-[³H]leucine there was very little radioactive albumin or Apo A-I in the blood. After this initial 15-min period, both nascent albumin and Apo A-I entered the blood and continued to accumulate until 30 min. Radioactive albumin reached a maximal level in the blood by 45 min and radioactive Apo A-I by 30 min.

Time of Intracellular Transport of Albumin and Apo A-I from RER to the Golgi Cell Fractions

To determine whether nascent Apo A-I and albumin are transported at the same rate from site of synthesis on the RER to the Golgi apparatus, young chickens were administered L-[³H]leucine intravenously and at different times the livers were removed, fractionated into the RER and Golgi cell fractions, and the time taken for radioactive albumin and Apo A-I to appear in these cell fractions were measured. At given times, the amounts of both albumin and Apo A-I were measured in the same animal. Pulse-labeled albumin reached maximal amounts in the RER between 3 and 9 min after the administration of L-[³H]leucine and between 9 and 25 min in the Golgi apparatus (Fig. 2A). By contrast, maximal Apo A-I radioactivity occurred at the same time (from 3 to 15 min) in both the RER and the Golgi cell fractions (Fig. 2B). There is an indication, at 3 min, that Apo A-I first enters the RER, but it also appears very rapidly in the Golgi cell fraction. The amounts of radioactive Apo A-I present in the RER and the Golgi cell fraction at 15 min suggests that Apo A-I is cleared from the RER before it is emptied from Golgi vesicles. These experiments indicate that although albumin and Apo A-I are secreted into the blood at similar rates (Fig. 1), nascent Apo A-I undergoes a much more rapid transport from the RER to the Golgi apparatus than does albumin.

Amino Acid Composition and NH₂-Terminal of Serum Apo A-I Obtained from Young Chickens

Because the NH₂-terminal amino acid sequence of serum Apo A-I, obtained from young chickens, has been reported to
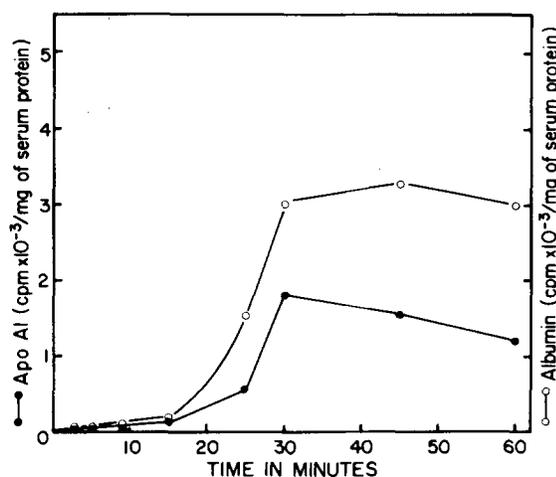

FIGURE 1   Time course of nascent Apo A-I and albumin secretion by chicken liver. Young chickens were injected intravenously with L-[4,5-³H]leucine. At specified times, the birds were killed and blood was collected. Radioactive Apo A-I and albumin were isolated from the serum by immunoprecipitation as detailed in Materials and Methods. The closed circles indicate the radioactivity in newly secreted Apo A-I and open circles that of albumin.
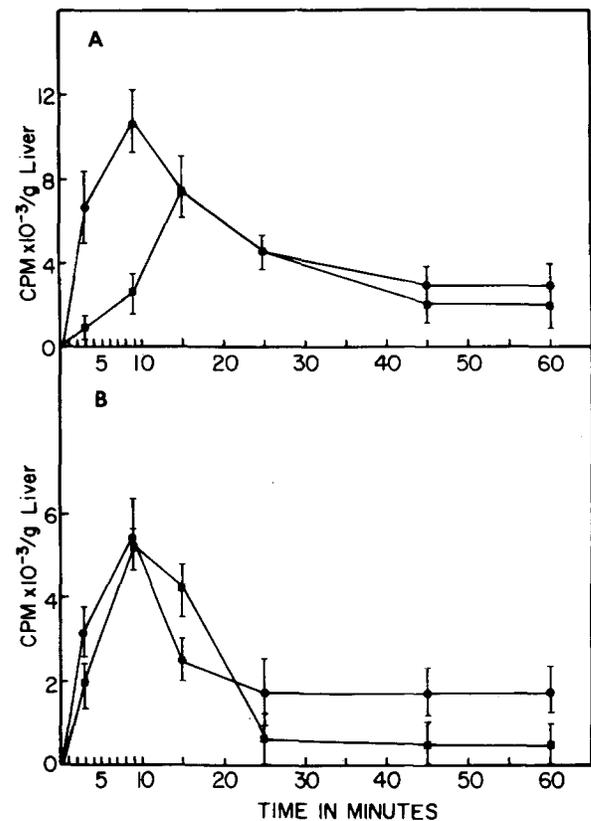

FIGURE 2   Time of intracellular transport of albumin and Apo A-I from RER to the Golgi apparatus. Young chickens were injected with L-[4,5-³H]leucine and at specified times the livers were removed and fractionated into RER and Golgi cell fractions. Radioactive Apo A-I and albumin were obtained by immunoprecipitation as described in Materials and Methods. The values given are the average of three experiments. A shows the albumin radioactivity while B shows that of Apo A-I. ●, RER; ■, Golgi cell fractions.

be different from that obtained from hens (21, 32), we isolated Apo A-I from young chicken serum HDL, determined its amino acid composition and the amino acid sequence of 20 NH₂-terminal residues, and compared it to that of Apo A-I obtained from hen, rooster, and human serums. Apo A-I from the serum of young chickens contained 248 amino acid residues per mol and the overall amino acid composition was very similar to that of hen (21), rooster (25), and human (2) Apo A-I with a few exceptions (Table I). There are only six residues of serine as compared to 13 for hen, 10 for rooster, and 14 for human Apo A-I. Young chicken serum Apo A-I also appeared to contain slightly higher amounts of glycine, which may be due to the fact that in the final stage of purification of Apo A-I by SDS PAGE, a glycine buffer was used.

Twenty cycles of automated Edman degradation of young chicken serum Apo A-I revealed the following amino terminal sequence: D.E.P.Q.T.P.L.D.R.I.R.D.M.V.D.V.T.L.E.T.[2] (Table II). This amino-terminal sequence is identical to that previously reported by Shackelford and Lebherz (32) for chickens and differs from that of Apo A-I from hen and humans. The reported NH₂-terminal sequences for hen serum Apo A-I is D.E.P.Q.P.E.L. (21) and for human serum Apo A-I is D.E.P.P.Q.S.P. (6).

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[2] In this paper a one-letter notation for Amino Acid Sequences as recommended by IUPAC-IUB Commission on Biochemical Nomenclature (J. Biol. Chem., 1968, 243:3557–3559) is used.

TABLE I. *Comparison of the Amino Acid Composition of Apo A-I from the Serum of Young Chicken, Hen, Rooster, and Human*

Amino acids	Young chicken	Hen (21)	Rooster (25)	Human (2)
Aspartic acid	21	20	20	21
Threonine	9	12	11	10
Serine	6	13	10	14
Glutamic acid	50	50	47	47
Proline	10	8	12	10
Glycine	17	5	3	10
Alanine	22	21	23	19
Valine	13	13	13	13
Methionine	5	4	5	4
Isoleucine	5	5	6	ND
Leucine	33	33	33	39
Tyrosine	6	5	7	7
Phenylalanine	6	4	5	6
Lysine	23	20	21	21
Histidine	1	1	1	5
Arginine	21	16	18	16
Tryptophan	ND	4	1	4
Half-cystine	ND	ND	ND	ND
Total	248	234	236	246
Molecular weight	27,000	26,674	26,000	28,331

The results, expressed as residues per mol, are averages of duplicate analyses of at least two different samples assuming a molecular weight of 27,000.

TABLE II. *Amino-Terminal Sequence Analysis of Young Chicken Serum Apoprotein A-I*

Sequenator cycle no.	PTH* amino acid identified	Yield
		(nmol)
1	Aspartic acid	22.6
2	Glutamic acid	20.8
3	Proline	2.4
4	Glutamine	32.7
5	Threonine	7.4
6	Proline	3.6
7	Leucine	3.9
8	Aspartic acid	21.7
9	Arginine	22.4
10	Isoleucine	5.1
11	Arginine	22.7
12	Aspartic acid	11.1
13	Methionine	8.0
14	Valine	3.6
15	Aspartic acid	7.9
16	Valine	2.6
17	Tyrosine	1.58
18	Leucine	5.0
19	Glutamic acid	12.8
20	Threonine	2.66

Approximately 225 μg lyophilized Apo A-I was dissolved in 10% glacial acetic acid and subjected to automated Edman degradation analysis as described in Materials and Methods. The PTH–amino acid residues were identified by high pressure liquid chromatography.
* *PTH*, phenylthiohydantoin.

Amino-Terminal Sequence of Nascent Intracellular and Secreted Apo A-I

Aspartic acid and proline occur at the amino-terminal portion of both chicken and human Apo A-I and we suspected that the pro-segment of chicken Apo A-I may be similar to that of the pro-segment of human Apo A-I. Therefore, in

order to determine whether or not the pro-segment of chicken Apo A-I is homologous to that of human Apo A-I and whether it is processed intracellularly, we administered to young birds, individually in separate experiments, the radioactive form of each of the amino acids present in the pro-segment of human Apo A-I, and of aspartic acid and proline. After 30 min, the blood and livers were collected and the radioactive Apo A-I present within the liver and that secreted into the blood was obtained by immunoprecipitation. The immunoprecipitates were subjected to twenty cycles of automated Edman degradation and the radioactivity in each cycle determined. The radioactive profile obtained in each cycle for the various radioactive amino acids used is shown in Fig. 3.
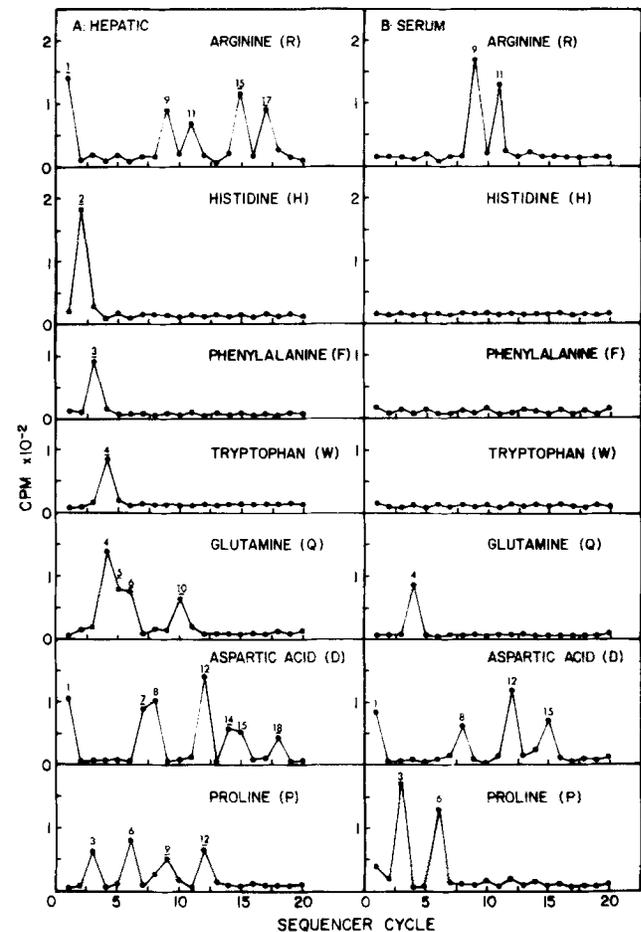

FIGURE 3   Radioactive amino acid sequence of intracellular and secreted Apo A-I. Young chickens were injected intravenously with the following individual radiolabeled amino acids; L-[5(n)-³H]arginine, L-[2,5-³H]histidine, L-[4-³H]phenylalanine, L-[5-³H]tryptophan, L-[G-³H]glutamine, L-[2,3-³H]aspartic acid, and L-[2,3-³H]-proline. After 30 min, the blood and livers were collected and radioactive Apo A-I present either intracellularly or in the serum was obtained by immunoprecipitation as described under Materials and Methods. To the isolated intracellular and secreted Apo A-I, 1 mg of sperm whale apomyoglobin was added and the samples were subjected to automated amino acid sequencing. The radioactivity in various cycle numbers is shown. The pertinent cycle numbers are given above the radioactive peaks. On the left side (A) the radioactive patterns obtained from intracellular Apo A-I are shown. On the right side (B) the radioactive patterns obtained from secreted Apo A-I are given. The cycle numbers in A are underlined when radioactivity would be expected if unprocessed pro-Apo A-I is present.

Intracellular radioactive Apo A-I contained two amino-terminal residues, arginine and aspartic acid, suggesting that it is a mixture of processed Apo A-I (containing NH_2-terminal aspartic acid) and of another form of Apo A-I, with NH_2-terminal arginine (Fig. 3A). Having determined the sequence of 20 NH_2-terminal amino acids of serum (processed) Apo A-I (Table II), any additional amino acids found in these positions should be due to the presence of pro-Apo A-I. Therefore the occurrence of arginine in cycles 1, 15, and 17, of histidine in cycle 2, of phenylalanine in cycle 3, or tryptophan in cycle 4, of glutamine in cycles 5, 6, and 10, of aspartic acid in cycles 7, 14, and 18, and of proline in cycles 9 and 12 indicates the following pro-hexapeptide extension: R.H.F.W.Q.Q. The occurrence of arginine in cycles 9 and 11, of glutamine in cycle 4, of aspartic acid in cycles 1, 8, 12, and 15, and of proline in cycles 3 and 6 identifies the other radioactive protein as processed Apo A-I since this assignment is identical to that obtained when serum Apo A-I was sequenced (Table II). NH_2-terminal sequence analysis of the secreted radioactive protein obtained from blood showed radioactive arginine in cycles 9 and 10, glutamine in cycle 4, aspartic acid in cycles 1, 8, 12,

A. NH_2-R H F W Q Q ↓ D E P Q T P L D R I R D M V
1 2 3 4 5 6   7 8 9 10 11 12 13 14 15 16 17 18 19 20

B. NH_2-D E P Q T P L D R I R D M V D V T L E T
1   3 4   6   8 9   11 12   15

Figure 4 NH_2-terminal amino acid sequence of intracellular pro-Apo A-I and secreted serum Apo A-I. The figure is based on the amino acid sequence of the amino-terminal portion of nascent Apo A-I obtained from the RER, Golgi cell fractions, and serum (Fig. 3 and 5). The arrow (↓) indicates the processing site of pro-Apo A-I to Apo A-I.

and 15, and proline in cycles 3 and 6 (Fig. 3B). This indicates that nascent Apo A-I in serum has the following NH_2-terminal sequence: D.—.P.Q.—.P.—.D.R.—.R.D.—.—.D.——. This sequence is identical to that of serum Apo A-I (Table II) and indicates that the processing is complete by the 30-min time point. A summary of the NH_2-terminal sequences of intracellular and secreted Apo A-I is presented in Fig. 4.

Cellular Sites of Processing of Pro-Apo A-I

To determine whether the processing of radioactive pro-Apo A-I occurs within the endoplasmic reticulum or the Golgi apparatus, young chickens were administered L-[2,3-^{3}H]proline (present in NH_2-terminal residues 3 and 6 of processed Apo A-I) or L-[G-^{3}H]glutamine (present in residues 5 and 6 of the pro-segment and in residue 4 of processed Apo A-I), and after 10 to 15 min the livers were fractionated into RER and Golgi cell fractions. Radioactive Apo A-I was isolated by immunoprecipitation from detergent-soluble RER and Golgi cell fractions and subjected to 20 cycles of automated amino acid sequence analysis. The results are given in Fig. 5. In the RER, glutamine radioactivity was found in cycles 5, 6, and 10 (Fig. 5A, upper panel), whereas in the Golgi cell fractions radioactive glutamine was present in cycles 5, 6, and 10 and also in cycle 4 (Fig. 5B, upper panel).

Proline radioactivity was found in cycles 9 and 12 for Apo A-I isolated from the RER (Fig. 5A, lower panel) and in cycles 3, 6, 9, and 12 for Apo A-I obtained from the Golgi apparatus (Fig. 5B, lower panel). This indicates that nascent Apo A-I in the Golgi region is a mixture of pro-Apo A-I and Apo A-I, whereas nascent pro-Apo A-I is the only Apo A-I form present in the RER.

Based on the amount of radioactivity in Edman degradation
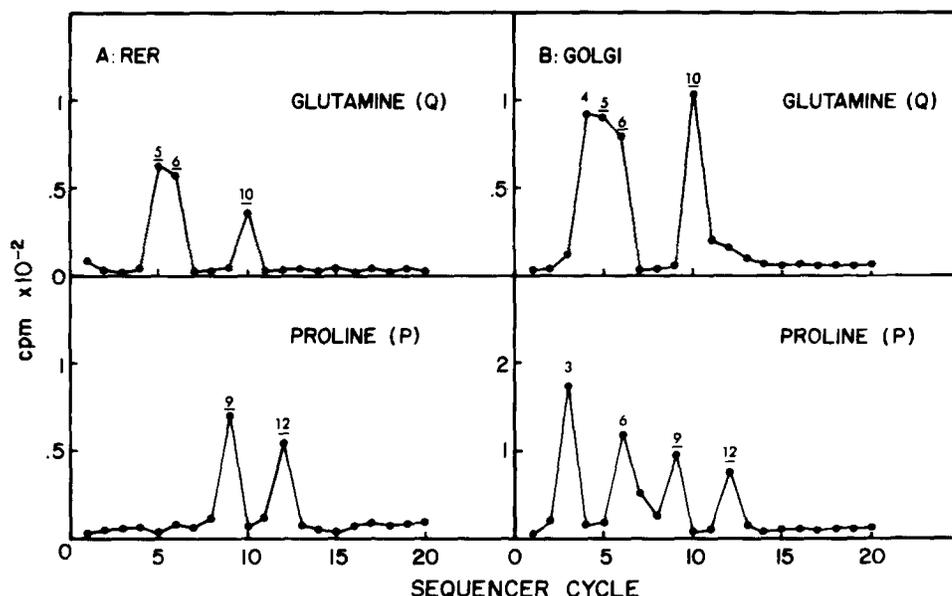
Figure 5   Location of L-[2,3-^{3}H]proline and L-[G-^{3}H]glutamine residues in Apo A-I obtained from the RER and Golgi cell fractions. Chickens were administered L-[^{3}H]proline or L-[^{3}H]glutamine for 10 to 15 min to label intracellular Apo A-I as described in Fig. 3. The RER and Golgi cell fractions from 28 g of liver were pooled and ruptured to release their contents as described in Materials and Methods. Radioactive nascent Apo A-I was isolated both from the RER and the Golgi contents by immunoprecipitation and subjected to automated amino acid sequencing as described in Fig. 3. Top panel shows the radioactive pattern obtained from Apo A-I labeled with L-[^{3}H]glutamine and bottom panel that with L-[^{3}H]proline. On the left hand side are the materials obtained from the RER and on the right hand side that from the Golgi cell fractions. The radioactive peaks are marked with the pertinent cycle numbers. The cycle numbers underlined are those in which radioactivity would be expected if unprocessed pro-Apo A-I is present.

TABLE III. *Percent Processing of Apo A-I*

	Total* intra-cellular	RER‡	Golgi apparatus‡	Serum*
	Percent radioactivity			
Pro-Apo-A-I	43.5 ± 12.8	100	49 ± 4.9	0
Apo-A-I	56.5 ± 12.8	0	51 ± 4.9	100

Individual radioactive amino acids were injected intravenously and at either 10 to 15 min (RER and Golgi apparatus) or 30 min (total intracellular and serum) nascent Apo A-I was isolated from the various cell fractions by immunoprecipitation and subjected to automated amino acid sequencing. The percent of pro-Apo A-I and Apo A-I was calculated from the yield obtained from the internal apomyoglobin standard and from the amount of radioactivity in each cycle when informative radiolabeled amino acids were used (Figs. 3 and 5).
* Calculations based on the averages from four different radioactive amino acids.
‡ Based on two experiments with different radioactive amino acids.

cycles which are distinctive for either pro-Apo A-I or for fully processed Apo A-I, and taking into account the recovery of derivatized amino acids (when using sperm whale apomyoglobin), the percent of pro-Apo A-I and processed Apo A-I in each sample was calculated. Intracellularly in the total liver 56.5% of pro-Apo A-I is processed to Apo A-I and a similar ratio (49%, pro-Apo A-I; 51% Apo A-I) was obtained in the Golgi cell fractions. By contrast, nascent Apo A-I in the RER is 100% in the pro-Apo A-I form; and in the serum 30 min after the administration of radioactive amino acid, 100% of the nascent Apo A-I is fully processed (Table III).

DISCUSSION

Since 1971, when Morgan and Peters studied the in vivo secretion of albumin and transferrin, it has been known that hepatic proteins travel through the intracellular secretory pathway at different rates (31). This variation in intracellular transit time is reflected in different secretion times for hepatic plasma proteins (27). The translocation of secretory proteins, from site of synthesis on the RER to the Golgi apparatus, is the step in which most variations occurs and this has led to the suggestion that receptors in the endoplasmic reticulum membrane select and regulate the transport of protein from the endoplasmic reticulum to the Golgi apparatus (29). Thus it is not surprising that nascent Apo A-I travels to the Golgi apparatus at a different rate than albumin. What is of interest however is the quickness with which Apo A-I enters the Golgi fractions and the length of time it is retained within Golgi apparatus-derived vesicles prior to secretion into the blood. Studies which have measured the rate of entry of nascent secretory hepatic proteins into the Golgi apparatus have shown that albumin usually leads and other proteins such as α1-antichymotrypsin and transferrin follow at a slower rate (29). The secretion of Apo A-I differs in that its transfer from RER to Golgi cell fraction is so rapid that it is difficult, by in vivo pulse-labeling methods, to measure its rate. Peak amounts of Apo A-I radioactivity are noticed in the RER and the Golgi cell fractions at the same time (between 3 and 15 min) and there is only a slight indication, at the earliest time points (3 min) that Apo A-I has entered the RER prior to the Golgi cell fraction (Fig. 2). By contrast, in the same animal, the stepwise progression of nascent albumin from RER to Golgi apparatus is clearly apparent. If albumin and Apo A-I are synthesized in similar locations on the RER and we accept the hypothesis that small carrier vesicles bud from the endoplasmic reticulum and carry the nascent proteins to the Golgi

apparatus (29), then our results imply that vesicles carrying nascent Apo A-I are immediately formed and transported to the Golgi region. Alternatively, the polysomes synthesizing Apo A-I may be located in specialized regions of the cytoplasm, in close juxtaposition to the Golgi apparatus, thus allowing faster translocation. Membrane-attached polysomes have been noticed close to Golgi apparatus (10). Another possibility is that, at the early time following pulse labeling, Apo A-I is not present in the Golgi apparatus but in some other cellular sub-fraction that co-fractionates with the Golgi cell fractions. This is unlikely, since the Golgi cell fractions (described in references 3 and 4) are devoid of RER vesicles. If the pulse-labeled Apo A-I is present in a vesicle which is not derived from the Golgi apparatus, it would have to be a specialized smooth endoplasmic reticulum compartment which does not contain nascent albumin, such as the postulated carrier vesicles which travel from the endoplasmic reticulum to the Golgi apparatus.

Our previous studies showed that nascent Apo A-I in the RER contain very little lipid and that most of the assembly of Apo A-I into lipoproteins occurs in the Golgi cell fraction (4). What determines when and how lipid–protein conjugation occurs is not known, but a mechanism must exist in the endoplasmic reticulum to protect nascent Apo A-I from binding to or from being inserted permanently into existing lipid particles. There may be a need, therefore, for Apo A-I to be quickly segregated within the endoplasmic reticulum and the haste by which it is transported to the Golgi apparatus may be in order to position it in the locale at which proper lipid–protein interactions may occur. The rapidity with which nascent Apo A-I enters the Golgi cell fraction is reminiscent of the rate at which pulse-labeled total membrane proteins enter this fraction (30).

Studies with rats and humans, using both liver and intestine, with either perfused tissues, organs, or cell culture, have shown that pro-Apo A-I is secreted into the blood and is then later processed, by the removal of an NH_2-terminal hexapeptide, to Apo A-I (9, 12, 13, 15, 38, 41). In young chickens the secretion of Apo A-I is clearly different. Pro-Apo A-I is the only form present in the RER, a near equal mixture of pro-Apo A-I and Apo A-I is found in the Golgi cell fraction, and 100% of the newly secreted apolipoprotein present in the serum is processed Apo A-I. These results may be interpreted in several ways. Young chickens may begin to process pro-Apo A-I to Apo A-I in the Golgi cell fraction and the processing is completed prior to secretion. In this case only processed Apo A-I is secreted into the blood. Another possibility is that, since there is a mixture of pro-Apo A-I and Apo A-I in the Golgi fraction, this mixture is secreted and pro-Apo A-I is then immediately converted by an extracellular enzyme to Apo A-I. We do not detect, however, at 30 min of secretion, any pro-Apo A-I in the circulating blood. A third possibility is that there is no processing intracellularly and that blood enzymes, present in the liver during homogenization may account for the presence of intracellular processed Apo A-I. It is unlikely however that these processing enzymes will penetrate the Golgi vesicles and not enter the vesicles derived from the RER. However we can not rule out the possibility that pro-Apo A-I is sequestered in the RER differently than in the Golgi apparatus or is in a conformation unfavorable to processing. A fourth possibility is that processing occurs extracellularly and that nascent Apo A-I is rapidly endocytosed by the liver. The method used in this study for preparation of

Golgi cell fractions has been shown to contain endosomes (24), and these endosomes could provide the 51% radioactive processed Apo A-I found in the Golgi cell fraction. This latter possibility is considered unlikely since the amount of processing in the Golgi cell fraction was measured between 10 and 15 min after the administration of radioactive amino acids, and at that time there was little or no radioactive Apo A-I secreted into the blood (see Fig. 1). The most likely possibility is that processing begins in the Golgi cell fraction and continues to occur in the terminal events of secretion. A metalloenzyme which converts human pro-Apo A-I to Apo A-I has been detected in plasma HDL, in mesenteric lymph, and in lymph chylomicrons (9). The source and exact nature of this extracellular enzyme have not been determined and we do not know whether or not a similar enzyme is present in chickens. The facts that the processing enzyme has been detected in lipoprotein particles and that, in chicken, processing occurs intracellularly in a Golgi cell fraction suggests that the processing enzyme could be a part of nascent HDL. The processing enzyme may be inserted into HDL during assembly and processing may occur at any subsequent stage of HDL secretion. This would be different to the processing of other secretory pro-proteins whose point of cleavage are usually marked by arginine residues. In these latter cases the processing enzyme is thought to reside in the Golgi membrane (36).

Paired glutamine residues, at the site of proteolytic processing, are not unique to pro-Apo A-I. The initial translation product of tropoelastin b mRNA contains a 24 amino acid NH₂-terminal peptide which, like the prosegment of Apo A-I, contains a gln–gln dipeptide at its carboxyterminus (23). Tropoelastin b, a major component of connective tissue, is an extracellular protein. It is not known, however, whether the paired glutamine residues mark the cleavage sites of a signal or a pro-sequence. If the paired glutamine residues mark the end of the signal sequence, then it could be assumed that cleavage occurs co-translationally. If, however, tropoelastin b also contains a prosegment, which is marked by the two glutamine residues, then cleavage may be either intracellular, as in chicken Apo A-I, or extracellular, as in rat and human Apo A-I.

Our studies show that the prosegments of chicken and human Apo A-I are both six amino acids in length with identical sequences including a pair of glutamines at the carboxy-terminal end. In addition, the NH₂-terminal portions of processed Apo A-I are similar, both commencing with asp. glu. pro...residues. Yet the pro-Apo A-I in human and rat, which presumably also travels through the Golgi apparatus, is not cleaved intracellularly to Apo A-I whereas that of chicken is processed. That pro-Apo A-I may be cleaved intracellularly in one species and not in another, and yet both have identical prosegments, is puzzling. The difference in processing between human, rat, and chicken may be a species difference, may be due to a difference in the mode of action of the processing enzyme, or may be due to the fact that only in chicken has processing been measured in vivo while in the human and rat, organ and cell culture methods have been used.

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